Language EuroNet Services

TRANSLATION
INTERPRETATION
A-V NARRATION
MULTILINGUAL TYPESETTING
LINGUISTIC CONSULTATION

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TO WHOM IT MAY CONCERN:

I, Eleonore A. Speckens, President of EuroNet Language Services Inc., located at 295 Madison Avenue, 45th Floor, New York, New York 10017, have a thorough knowledge of the German and English languages and hereby certify that the attached patent application DE 19746874.8 in the English language is the true and accurate translation of the attached patent application DE 19746874.8 in the German language.

On this, the thirtieth day of November, 1999, New York, NY 10017

Eleonore A. Speckens

Sworn before me on this date

12/1/99

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File No.: 197 46 874.8-44
Your ref.: PA044-K-3007
App. No.: 5461391
QIAGEN GmbH

Qiagen GmbH
Attn. Dr. Rainer Wessel
4 Max-Vollmer-Strasse
40724 Hilden

Bibliography Report

IPC Hk1	C07H 21/00		File No. 197 46 874 8-44
IPC Nk1	C07H 1/06	C07H 1/08	G01N 33/50
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App.	10/23/1997		
Des.	Process for isolation and purification of nucleic acids on hydrophobic surfaces, in particular with the use of hydrophobic membranes.		
App. No.	5461391 QIAGEN GmbH, 40724 Hilden, DE		
Inv.	Dr. Helge Bastian, 40597, Duesseldorf, DE; Simone Gauch, 40597 Duesseldorf, DE; Dr. Uwe Oelmueller, 40699 Erkrath, DE P		

The application will probably be published on April 29, 1999.

The technical preparations pursuant to Article 32 Section 4 PatG [Patent Law] were completed 8 weeks ago.
Publication of the disclosure document will be dispensed with only if, more than 8 weeks prior to the publication date given above, the application is withdrawn or rejected or is to be deemed withdrawn (Article 32 Section4 PatG.).

Note:
No further requirements

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Qiagen GmbH
Attn. Dr. Rainer Wessel
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197 46 374.8

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(2) Symbol of the applicant/representative (max. 20 characters)
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(3) The recipient in box (1) is the
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(5) NA
(6) Designation of the invention *(if too long, use separate page in duplicate)*
Process for isolation and purification of nucleic acids on hydrophobic surfaces, in particular with the use of hydrophobic membranes.

(7) Other applications
Application for examination - Examination of the application (Article 44 Patent Law)
Examination proceeding

(10) Fee payment in the amount of DM 520.00
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(11) Annexes (Annexes 3-7 in triplicate)

Preliminary fax on 10/23/97

3. 1 summary (with figures, if necessary)
4. 14 pages of description
6. 6 pages of patent claims
   50 patent claims 11/14/97 [signature] QIAGEN GmbH
   4 Max-Volmer-Strasse
7. 1 sheet of figures (12) Signature(s)
   Dr. Metin Colpan (Manager)

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(6) Designation of the invention *(if too long, use separate page in duplicate)*
Process for isolation and purification of nucleic acids on hydrophobic surfaces, in particular with the use of hydrophobic membranes.

(7) Other applications
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Examination proceeding - Examination process

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Process for the isolation and purification of nucleic acids on hydrophobic surfaces - in particular, using hydrophobic membranes

This invention concerns a new process for the isolation and purification of nucleic acids on hydrophobic surfaces, using hydrophobic membranes or membranes whose surfaces have been made hydrophobic.

The isolation and purification of nucleic acids from biological and clinical sample material is of crucial importance for fields of work in which operating techniques based on nucleic acids are employed, or in which technologies based on nucleic acids are actually the key to access. Examples include paternity analysis, tissue classification, identification of hereditary diseases, genome analysis, molecular diagnostics, determination of infectious diseases, animal and plant breeding, transgenic research, basic research in biology and medicine, as well as numerous related areas. In general, a difficulty is encountered in preparing biological or clinical sample materials in such a manner that the nucleic acids contained in them can be used directly in the analytic procedure in question.

The state of the art already includes many processes for the purification of DNA. For example, we know how to purify plasmid DNA for the purpose of cloning - and other experimental processes as well - according to the method of Birnboim [Methods in Enzymology 100 (1983) 243]. In this process, a cleared lysate of bacterial origin is exposed to a cesium chloride gradient and centrifuged for a period of 4 to 24 hours. This step is usually followed by the extraction and precipitation of the DNA. This process is associated with the disadvantage that it is very apparatus-intensive and, on the other hand, it takes a great deal of time, is cost-intensive and cannot be automated.

Other techniques in which cleared lysates are used to isolate DNA are based on ion-exchange chromatography [Colpan et al., J. Chromatog. 296 (1984) 339] and gel filtration [Moreau et al., Analyt. Biochem. 166 (1987) 188]. These processes are offered primarily as alternatives to the cesium chloride gradients, but these require a very apparatus-intensive solvent supply system and a precipitation of the DNA fractions is necessary, since these usually contain salts in high concentrations and are extremely diluted solutions.

Marko et al. [Analyt. Biochem., 121 (1982) 382] and Vogelstein et al. [Proc. Nat. Acad. Sci. 76 (1979) 615] have found that if the DNA from extracts containing nucleic acids is exposed to high concentrations of sodium iodide or sodium perchlorate, only DNA will adhere to glass scintillation tubes, fiberglass membranes or fiberglass sheets that have been finely ground by mechanical means, while RNA and proteins do not. The DNA that has been bound in this manner can be eluted, for

example, with water.

In the various teachings included in the state of the art, it is also generally stated that all processes for the purification of nucleic acids that are based on the principle of solid-phase extraction have recourse to the use of hydrophilic surfaces - such as modified silica materials - (for example, DEAE anionic-exchange chromatography, silica membranes or silica particles).

In the state of the art, it is generally noted that the surfaces must definitely exhibit a hydrophilic character - as exists, for example, in the case of silica gel - if these are to be suitable for the immobilization of nucleic acids. On the other hand, there is only one reference in the state of the art to the use of hydrophobic surfaces.

For example, in WO-A-87/06621, the immobilization of nucleic acids on a PVDF membrane is described. However, the nucleic acids bound to the PVDF membrane are not eluted in the next step; instead the membrane, together with all the bound nucleic acids is transferred directly into a PCR reaction. Finally, in this international patent application and in the other literature, it is stated that hydrophobic surfaces or membranes must in general be wetted beforehand with water or alcohol, in order to be able to immobilize the nucleic acids with yields that are somewhat satisfactory.

On the other hand, for a number of modern applications - such as, for example, the PCR, reversed transcription PCR, SunRise, LCX-branched-DNA, NASBA, or TaqMan technologies and similar real-time quantification techniques for PCR, it is absolutely necessary to be able to release the nucleic acids directly from the solid phase. In this connection, WO-A-87/06621 teaches us that, while the nucleic acids can indeed be recovered from the membranes used in the process, this recovery is fraught with problems and is far from suited to the quantitative isolation of nucleic acids. In addition, the nucleic acids obtained in this manner are, comparatively, extremely dilute -a circumstance that makes subsequent isolation and concentration steps absolutely necessary.

For the reasons stated above, the processes known from the state of the art do not constitute - particularly with regard to automation of the process for obtaining nucleic acids - a suitable starting point for an isolation of nucleic acid that is as simple and quantitative as possible from the point of view of process engineering.

The purpose of this invention is therefore to overcome the disadvantages of the processes known from the state of the art for the isolation of nucleic acids and to provide a process which is capable of being almost completely automated without substantial additional technical expenditure.

Another purpose of this invention is, in particular, to bind nucleic acids to an immobile phase - especially to a membrane - in such a manner that in a subsequent reaction step they can be released immediately from this phase and, if desired, used in other applications, such as, for example, restrictive digestion, RT, PACR, RT-PCR or NASBA, as well any other suitable analytic or enzymatic reaction named above.

These purposes are accomplished according to the invention through the use of porous hydrophobic membranes, to which the nucleic acids from the sample containing them can be bound in a simple manner and then be released in an equally simple process, with the simple procedure dictated by the invention making it possible, in particular, to fully automate the process.

According to this invention, a sample containing nucleic acids is defined as a sample or a sample preparation that contains nucleic acids which can serve as suitable educts for *in vitro* transcriptions, PCR reactions, NASBA reactions or cDNA syntheses of, for example, plasma, body fluids - such as blood, sputum, urine, feces - cells, serum, smears, tissue samples of every kind, plants and parts of plants, bacteria, viruses, yeasts, etc., as, for example, disclosed in European Patent Application No. 95909684.3, to whose content reference is hereby made, or also free nucleic acids.

In the process according to the invention, the sample containing nucleic acids described above is placed in a solution which contains suitable salts or alcohol(s), then, if necessary, renders the preparation soluble and mixes it, and the mixture so obtained is conducted through a hydrophobic membrane by means of a vacuum, through centrifuging, or by positive pressure, capillary force or any other suitable procedure.

Suitable salts for the immobilization of nucleic acids on hydrophobic membranes include salts of the alkaline or alkaline earth metals with mineral acids, in particular alkaline or alkaline-earth halogenides or sulfates, with the halogenides of sodium or potassium or magnesium sulfate being especially preferred.

Also suited for the conduct of the process according to the invention are salts of mono- or polybasic acids or polyfunctional organic acids with alkaline or alkaline-earth metals. These include, in particular, salts of sodium, potassium or magnesium with organic dicarboxylic acids - such as oxalic, malonic or succinic acid - or with hydroxy- or polyhydroxycarboxylic acids - for example, preferably, with citric acids.

The use of so-called chaotropic agents has proved to be especially effective. Chaotropic substances are capable of disturbing the three-dimensional structure of hydrogen bonds. As is generally known, this process also weakens the intramolecular binding forces that participate in forming the spatial structures - including primary, secondary, tertiary or quaternary structures - in biological molecules. Chaotropic agents of this kind are known to the expert from the state of the art (Rompp, Lexikon of Biotechnologie, published by H. Dellweg, R.D. Schmid and W.E. Fromm, Thieme Verlag, Stuttgart 1992).

The preferred chaotropic substances for use with this invention are, for example, salts from the trichloroacetate, thiocyanate, perchlorate or iodide group or from guanidine hydrochloride and urea.

The chaotropic substances are used in a 0.01- to 10-molar aqueous solution, preferably in a 0.1- to

7-molar aqueous solution and most preferably in a 0.2- to 5-molar aqueous solution. The chaotropic agents mentioned above can be used alone or in combinations. In particular, a 0.01- to 10-molar aqueous solution, preferably a 0.1- to 7-molar aqueous solution and most preferably a 0.2- to 5-molar aqueous solution of sodium perchlorate, guanidinium hydrochloride, guanidinium-iso-thyocyanate, sodium iodide or potassium iodide.

The suitable alcohols for the conduct of the process according to the invention include, first of all, all the hydroxyl derivatives of aliphatic or acyclic saturated or unsaturated hydrocarbons. It is initially unimportant whether the compound in question contains one-, two, three or more hydroxyl groups - such as polyvalent C1-C5 alkanols, including ethylene glycol, propylene glycol or glycerine.

In addition, the alcohols that can be used in the invention include the sugar derivatives - the so-called aldites - as well as the phenols, such as polyphenols.

Among the hydroxyl compounds mentioned above, the C1-C5 alkanols, such as methanol, ethanol, n-propanol, tertiary butanol and the pentanols are especially preferred.

Hydrophobic materials for purposes of this invention are those materials or membranes that, due to their chemical character do not penetrate water - or vice versa - cannot remain in it.

For purposes of this invention, a hydrophobic surface - preferably a membrane - is understood to be any microporous dividing layer that displays a hydrophobic character. In the case of a membrane, the hydrophobic surface consists of a film made from a polymer material, preferably containing polar groups, such as ester, carbonyl or amide groups. As an alternative, it is conceivable to mount the polar group on a surface when the hydrophobic coating agent is transferred to it, even if the surface itself has no polar groups, if this is desired.

In another embodiment of the process according to the invention, the concept of "surface" in the broader sense includes a layer of particles or a granulate or even fibers with hydrophobic characteristics, which consist of the materials originally forming the membranes.

Membranes preferred for the purposes of this invention are those consisting of a hydrophilic basic material and which are made hydrophobic by an appropriate chemical post-treatment, known from the state of the art, such as, for example, hydrophobisized nylon membranes that can be obtained commercially.

For the purposes of this invention, hydrophobisized membranes include, in general, those membranes which may or may not have been hydrophilic to begin with and are coated with the hydrophobisized agents mentioned below. Hydrophilic coating agents of this kind cover hydrophilic substances with a thin layer of hydrophobic groups, such as fairly long alkyl chains or siloxane groups. Suitable hydrophobic coating agents are known in great number from the state of the art;

for purposes of the invention, these include paraffins, waxes, metallic soaps etc., if necessary with additives of aluminum or zirconium salts, quarternary organic compounds, urea derivatives, lipid-modified melamine resins, silicones, zinc-organic compounds, glutaric dialdehyde and similar compounds.

In addition, the hydrophobic membranes that can be used for purposes of the invention are those that have been hydrophobisized and whose basic material contains polar groups. According to these criteria, for example, materials from the following groups - particularly hydrophobisized ones - are suitable for use with the invention.

Nylon, polysulfones, polyether sulfones, polycarbonates, polyacrylates and acrylic acid copolymers, polyurethanes, polyamids, polyvinylchlorides, fluorocarbonates, polytetrafluoroethylene, polyvinylene difluoride, ethylene tetrafluoroethylene, polyethylene-chlorotrifluoroethylene copolymerisate or polyphenylene sulfide, and cellulose-mix esters or nitrocelluloses as well as hydrophobisized optical fiber membranes, with hydrophobisized nylon membranes being especially preferred.

The membranes that are used in the process according to the invention have, for example, a pore diameter of 0.05 to 20 μm, preferably 0.2 to [omission] μm and most preferably 0.45 to 5.0 μm.

For washing buffers, the salts or alcohols, phenols or polyphenols, described above can be used. The temperatures in the washing step will usually be within the range from 10 to 30° C; higher temperatures can also be used successfully.

Suitable eluting agents for the purposes of the invention are water or aqueous salt solutions. As salt solutions, buffer solutions that are known from the state of the art are used, such as morpholinopropane sulfonic acid (MOPS), Tris (hydroxymethyl) aminomethane (TRIS), 2-[4-(2-hydroxyethyl) piperazino] ethane sulfonic acid (HEPES) in a concentration from 0.001 to 0.5 moles/liter, preferably 0.01 to 0.2 moles/liter, most preferably 0.01 to 0.05 molar solutions. Also preferred for use are aqueous solutions of alkaline or alkaline-earth metal salts - in particular, their halogenides - including 0.001 to 0.5 - preferably 0.01 to 0.2 molar, most preferably 0.01 to 0.05 molar - aqueous solutions of sodium chloride, lithium chloride, potassium chloride or magnesium chloride. Also preferred for use are solutions of salts of the alkaline or alkaline-earth metals with carboxylic or dicarboxylic acids - oxalic acid or acetic acid - in the range of concentrations mentioned above - for example, 0.001 to 0.5 - preferably 0.01 to 0.2 molar, most preferably 0.01 to 0.05 molar solutions of sodium acetate or oxalate in water.

Water is by far the best eluting agent.

The elution for purposes of the invention can be accomplished by positive pressure, vacuum, centrifuging or capillary forces.

With regard to the individual steps, the process according to the invention can be performed as follows:

The lysate of the sample used for the recovery of the nucleic acids or the originally free nucleic acid(s) is/are pipetted, for example, in a (plastic) column, in which the hydrophobic membrane is fastened - for example, on the floor. It is more efficient if the membrane is fastened to a grid, which serves as a mechanical support. The lysate is then conducted through the membrane, which can be achieved by applying a vacuum at the outlet of the column. The transport can also be accomplished by applying positive pressure to the lysate. In addition - as mentioned above - the transport of the lysate can take place by centrifuging or by the effect of capillary forces. The latter can be produced, for example, with a sponge-like material which is introduced below the membrane, in contact with the lysate or filtrate.

The advantage of such an arrangement consists of a simple, reliable and handy possibility for disposing of the filtrate - in this case, only the sponge, which is now more or less saturated with the filtrate, need be exchanged. It should be clear at this point that the column can be operated continuously or batch-wise and that both these modes of operation can be fully automated, until the hydrophobic membrane is completely saturated with nucleic acid. The final step is the elution of the nucleic acid, which can, for example, be pipetted or lifted from the membrane or suctioned, pressured or centrifuged through the abovementioned layer of particles, granulate of fibers made of hydrophobic membrane materials.

The capture of fractions that contain the desired nucleic acids in highly diluted solutions and require a subsequent concentration becomes completely unnecessary with the process according to the invention; instead, the desired nucleic acids are obtained in solutions containing little or no salt in very small volumes, which is of great advantage for all molecular biological analytic procedures, since these demand pure nucleic acids in the smallest possible volumes with a simultaneously high concentration.

The final process step - as well as the preparation of the membrane or column according to the invention for the subsequent isolation of the nucleic acid - can easily be fully automated, since the nucleic acids that have been completely eluted can be easily removed from the membrane from the top by vacuum or pipetting.

This invention also offers the advantage that the space above the membrane can be used as a reaction chamber. Thus, for example, it is possible, after the nucleic acid obtained by means of the process according to the invention has been isolated and eluted, to subject it to a molecular biological application - such as restrictive digestion, RT, PCR, RT-PCR, NASBA or enzyme reactions, to bind the nucleic acids produced from these reactions again to the hydrophobisized membrane, to wash them and then to eluate them, isolate them or analyze them - for example, by spectroscopy, fluorometry or similar measuring processes by means of the process according to the invention.

The invention described above is illustrated in the following examples. Various other embodiments of the process will be obvious to the expert from the preceding description and the examples. It is, however, expressly stated that these examples and the description that accompanies them are intended solely for the purpose of illustration and are not to be regarded as a limitation of the scope of the invention.

Example 1

Isolation of total RNA from HeLa cells

Commercially available nylon membranes (for example, a material from MSI: Magna SH with a pore diameter of 1.2μm or a material from Pall GmbH: Hydrolon with a pore diameter of 1.2 μm) which have been made hydrophobic by means of a chemical post-treatment are placed in a plastic column. The membranes are placed on a polypropylene grid which serves as a mechanical support. The membranes are fixed in the plastic column with a ring.

The column prepared in this manner is connected by means of a Luer connection to a vacuum chamber. All the isolation steps are conducted through the application of a vacuum.

For the isolation, 5 x 10^5 HeLa cells are pelletized by centrifugation. The cells are lysated by the addition of 150 μl of a commercial guanidium isothiocyanate buffer - for example RLT buffer from QIAGEN - in a manner thoroughly familiar from the state of the art. Lysis is promoted by roughly mixing by pipetting or vortexing over a period of about 5 s. Then 150 μl of 70% ethanol are added and mixed in by pipetting or by vortexing over a period of about 5 s.

The lysate is then pipetted into the plastic column and suctioned through the membrane by evacuating the vacuum chamber. Under the conditions thus created, the RNA remains bound to the membrane. Next, washing takes place with a first commercial washing buffer containing guanidium isothiocyanate - for example, with the RW1 buffer of QIAGEN - and, after that, with a second washing buffer containing TRIS or TRIS and alcohol - for example, with the RPE buffer of QIAGEN. The washing buffers in each case are suctioned through the membrane by evacuation of the vacuum chamber. After the final washing step, the vacuum is maintained for a period of about 10-min, in order to dry the membrane, after which the vacuum chamber is switched off.

For the elution, 70μl RNase-free water is pipetted onto the membrane in order to release the purified RNA from the membrane. After incubation for one minute at a temperature in the range from 10 to 30° C, the eluate is pipetted from the membrane from the top and the elution step is repeated in order to make sure that the elution is complete.

The volume of isolated total RNA obtained in this manner is then determined by spectrophotometric measurement of the light absorption with a wavelength of 260 nm. The ratio between the absorbance

values at 260 and 280 nm gives an estimate of RNA purity (see Fig. 1: Total RNA (isolated by Hydrolon 1.2).

The results of the two isolations with hydrophobic nylon membranes (Nos. 1 and 2) are shown in Table 1, compared with experiments in which for [omission] a hydrophilic nylon (Nyaflo) (No. 3) and a silica membrane were used. The values reported in the table provide convincing support for the impressive isolation yield and separation effect of the hydrophobic materials used in accordance with the invention.

Table 1. RNA yield and purity of the total RNA isolated in accordance with Example 1

No.	Type of membrane	Yield of Total RNA (µg)	E260/E280
1	Magna SH 1.2µm (hydrophobic nylon)	6.0	1.97
2	Hydrolon 1.2µm (hydrophobic nylon)	7.1	2.05
3	Nyaflo (hydrophilic nylon)	<0.2	Not determined
4	Hydrophilic silica membrane	<0.2	Not determined

The isolated RNA can also be analyzed on agarose gels that have been stained with ethidium bromide. For this purpose, for example. 1.2% formaldehyde agarose gels are assembled. The result is shown in Figure 2.

In Figure 2, Lane 1 is a total RNA that was isolated by means of a hydrophobic nylon membrane from Magna SH with a pore diameter of 1.2µm.

Lane 2 is a total RNA that was isolated by means of a hydrophobic nylon membrane from Hydrolon with a pore diameter of 1.2µm.

Lane 3 represents the chromatogram of a total RNA that was isolated by means of a silica membrane.

In each case, 50 µl of the total RNA isolate was analyzed.

Fig. 2 provides convincing evidence that when a silica membrane is used, no measurable proportion of the total RNA can be isolated.

Example 2

Isolation of total RNA from mouse liver

In this example, the lysate and washing solutions are conducted through the hydrophobic membrane by centrifugation, and the RNA is eluted by removing the residue from the top.

5 mg of mouse liver, deep-frozen at -80° C are placed in a 2 ml reaction apparatus loaded with 350 µl of a commercially available lysis buffer containing guanidium isothiocyanate (for example, RLT buffer from QIAGEN and homogenized by means of an electric homogenizer for a period of 30 seconds. Next. 350 µl of 70% ethanol are added to the lysate and mixed for a period of 5 seconds by pipetting or by vortexing. Three lysates of this kind are then each transferred into a plastic column containing a 1.2 µm Magna SH hydrophobic nylon membrane, a hydrophobic Hydrolon membrane and - for purposes of comparison -optical-fiber membranes of hydrophilic silica, which are placed in a 2 ml receptacle. The lysate is in each case conducted through the membrane by centrifugation for a period of 15 s at 10,000 x g, and the RNA is left adhering to the membranes according to the values shown in Table 2.

The flowthrough product is thrown away in each case, and the column is again placed in the receptacle. The membranes are then washed once with a commercially available washing buffer (for example, the RW1 buffer from QIAGEN) and twice with another commercially available washing buffer containing guanidium isothiocyanate (for example, the RPE buffer from QIAGEN), with the washing buffer being conducted through the membranes in each case by centrifugation and the last washing step taking place at 18,000 x g for a centrifugation time of 2 minutes, in order to dry the membrane.

For the elution, 70 µl of RNase-free water is transferred onto the membrane, in order to release the purified RNA from it. After incubation for a period of one minute at 10 - 30° C, the eluates are removed from the top - that is, from the residue on the membrane - by means of a pipette. The elution step is then repeated once again.

The volume of isolated total RNA is determined by spectrophotometric measurement of the light absorption at 260 nm. The ratio between the absorbance values at 260 and 280 nm gives an estimate of RNA purity.

Table 2. RNA yield and purity of the total RNA isolated in accordance with Example 2

No.	Type of membrane	Yield of Total RNA (µg)	E260/E280
1	Magna SH 1.2µm (hydrophobic nylon)	15	1.92
2	Hydrolon 1.2µm (hydrophobic nylon)	14	2.05

3	Glass fiber membrane (Hydrophilic silica)	1.7	Not determined

Example 3

Isolation of free RNA by binding the RNA to hydrophobic membranes by means of various salt-alcohol mixtures. In this example, the lysate and washing solutions are conducted through the hydrophobic membrane by applying a vacuum.

Hydrophobic nylon membranes (for example, 1.2 mm Hydrolon from the Pall Company) are introduced into plastic columns that are connected to a vacuum chamber, in a manner similar to that of Example 1.

100 µl of an aqueous solution containing total RNA is mixed, by pipetting, with, respectively, 350µl of a commercially available lysis buffer containing guanidium isothiocyanate (for example, the RLT buffer from QIAGEN), 350 µl of 1.2 M sodium acetate solution, 350 µl 2 M sodium chloride solution and 350 µl of 4 M lithium chloride solution, respectively, and mixed by pipetting.

Next, 250 µl of ethanol is added to each mixture and mixed in, likewise by pipetting. After that, the solutions containing RNA are transferred into the plastic columns and suctioned through the membrane by evacuating the vacuum chamber. Under the conditions described, the RNA remains bound to the membranes. The membranes are then washed, as described in Example 1.

Finally, the RNA - also as described in Example 1 - is removed from the membrane by pipetting from the top.
The volume of isolated total RNA is determined by spectrophotometric measurement of the light absorption at 260 nm. The ratio between the absorbance values at 260 and 280 nm gives an estimate of RNA purity.

Table 3. Isolation of free RNA by binding the RNA to hydrophobic membranes by means of various salt-alcohol mixtures

No.	Salt-alcohol mixture	Yield of Total RNA (µg)	E260/E280
1	Qiagen RLT buffer	9.5	1.92
2	0.6 M sodium acetate / 35% ethanol	8.5	1.98
3	1.0 M sodium chloride / 35% ethanol	7.9	1.90

4	2 M lithium chloride /35% ethanol	4.0	2.01

Patent Claims

1. Process for the isolation of nucleic acids on hydrophobic surfaces, characterized by the fact that it includes the following steps:

- immobilization of the nucleic acids on a hydrophobic surface
- if desired, washing of the immobilized nucleic acids with a washing buffer
- release of the immobilized nucleic acids from the surface
- if desired, elution.

2. Process according to Claim 1, characterized by the fact that the hydrophobic surface is a membrane.

3. Process according to Claim 2, characterized by the fact that the hydrophobic membrane is made of a polymer with polar groups.

4. Process according to Claims 1 to 3, characterized by the fact that aqueous solutions of salts of the alkaline or alkaline-earth metals with mineral acids are used to immobilize the nucleic acids.

5. Process according to Claim 4, characterized by the fact that alkaline or alkaline-earth halogenides or sulfates are used to immobilize the nucleic acids.

6. Process according to Claim 5, characterized by the fact that halogenides of sodium or potassium or magnesium sulfate are used to immobilize the nucleic acids.

7. Process according to Claims 1 to 3, characterized by the fact that aqueous solutions of salts of mono- or polybasic or polyfunctional organic acids with alkaline or alkaline-earth metals are used to immobilize the nucleic acids.

8. Process according to Claim 7, characterized by the fact that aqueous solutions of salts of sodium, potassium or magnesium with organic dicarboxylic acids are used to immobilize the nucleic acids.

9. Process according to Claim 8, characterized by the fact that the organic dicarboxylic acids are oxalic acid, malonic acid or succinic acid.

10. Process according to Claim 7 characterized by the fact that aqueous solutions of salts of sodium or potassium with a hydroxy- or polyhydroxycarboxylic acid are used to immobilize the nucleic acids.

11. Process according to Claim 10, characterized by the fact that the polyhydroxycarboxylic acid is citric acid.

12. Process according to Claims 1 to 3, characterized by the fact that, if desired, additional chaotropic agents are used to immobilize the nucleic acids.

13. Process according to Claim 12, characterized by the fact that the chaotropic agent is a salt from the group of trichloroacetates, thiocyanates, perchlorates iodides or guanidine hydrochloride, guanidine isothiocyanate or urea.

14. Process according to Claim 12 or 13, characterized by the fact that 0.01 molar to 10 molar aqueous solutions of the chaotropic agents are used, alone or in combination with other salts, to immobilize the nucleic acids.

15. Process according to Claim 14, characterized by the fact that 0.1 molar to 7 molar aqueous solutions of the chaotropic agents are used, alone or in combination with other salts, to immobilize the nucleic acids.

16. Process according to Claim 15, characterized by the fact that 0.2 molar to 5 molar aqueous solutions of the chaotropic agents are used, alone or in combination with other salts, to immobilize the nucleic acids.

17. Process according to any of Claims 12 to 16, characterized by the fact that an aqueous solution of sodium perchlorate, guanidium hydrochloride, guanidium isothiocyanate, sodium iodide or potassium iodide is used to immobilize the nucleic acids.

18. Process according to Claims 1 - 3, characterized by the fact that hydroxyl derivatives of aliphatic or acyclic saturated or unsaturated hydrocarbons are used to immobilize the nucleic acids.

19. Process according to Claim 18, characterized by the fact that C1-C5 alkanols are used as the hydroxyl derivatives.

20. Process according to Claim 19, characterized by the fact that methanol, ethanol, n-propanol, tertiary butanol or pentanols are used as the C1-C5 alkanols.

21. Process according to Claim 18, characterized by the fact that an aldite is used as the hydroxyl derivative.

22. Process according to any of Claims 1 to 3, characterized by the fact that a phenol or polyphenol is used to immobilize the nucleic acids.

23. Process according to Claims 1 to 3, characterized by the fact that hydrophilic membranes with

a hydrophobisized surface are used to immobilize the nucleic acids.

24. Process according to any of Claims 1, 3 or 23, characterized by the fact that the hydrophilic surface or the membrane is made of nylon, a polysulfon, polyether sulfon, polycarbonate, polyacrylate, as well as an acrylic acid copolymer, polyurethane, polyamid,

25. Process according to Claim 24, characterized by the fact that the surface or membrane consists of hydrophobisized nylon.

26. Process according to any of Claims 23 - 25, characterized by the fact that the membrane is coated with a hydrophobic coating agent from the group of paraffins, waxes, metallic soaps, if desired with the addition of aluminum or zirconium salts, quaternary organic compounds, urea derivatives, lipid-modified melamine resins, silicones or zinc-organic compounds or with glutaric dialdehyde.

27. Process according to Claims 1 - 3, characterized by the fact that a salt solution or a buffer solution according to any of Claims 4 - 22 is used to wash the immobilized nucleic acids.

28. Process according to Claims 1 - 3, characterized by the fact that an aqueous salt or buffer solution is used for the elution of the nucleic acids.

29. Process according to Claim 28, characterized by the fact that the buffer solution contains one or more substances from the morpholinopropane sulfonic acid (MOPS), TRIS(hydroxymethyl) aminomethane (TRIS), 2-[4-(2-hydroxyethyl)-1-piperazin] ethane sulfonic acid (HEPES) groups.

30. Process according to Claim 28 or 29, characterized by the fact that the buffer substance is used in an aqueous solution with a concentration in the range from 0.001 to 0.5 mole/liter.

31. Process according to Claim 30, characterized by the fact that the buffer substance is used in an aqueous solution with a concentration in the range from 0.01 to 0.2 mole/liter.

32. Process according to Claim 31, characterized by the fact that the buffer substance is used in an aqueous solution with a concentration in the range from 0.01 to 0.05 mole/liter.

33. Process according to Claim 28, characterized by the fact that an aqueous solution of an alkaline or alkaline-earth metal halogenide is used as the salt solution.

34. Process according to Claim 33, characterized by the fact that an aqueous solution of sodium chloride, lithium chloride, potassium chloride or magnesium chloride is used as the salt solution.

35. Process according to Claim 33 or 34, characterized by the fact that the salt is used in the aqueous solution in a concentration ranging from 0.001 to 0.5 mole/liter.

36. Process according to Claim 35, characterized by the fact that the salt is used in the aqueous solution in a concentration ranging from 0.01 to 0.2 mole/liter.

37. Process according to Claim 36, characterized by the fact that the salt in the aqueous solution is used in a concentration ranging from 0.01 to 0.05 mole/liter.

38. Process according to Claim 28, characterized by the fact that an aqueous solution of a salt of an alkaline or alkaline-earth metal with a carboxylic or dicarboxylic acid is used as the salt solution.
39. Process according to Claim 38, characterized by the fact that the carboxylic acid is acetic acid and the dicarboxylic acid is oxalic acid..

40. Process according to Claim 39, characterized by the fact that the salt is sodium acetate or sodium oxalate.

41. Process according to any of Claims 38, 39 or 40, characterized by the fact that the salt is present in an aqueous solution in a concentration ranging from 0.001 to 0.5 mole/liter.

42. Process according to Claim 41, characterized by the fact that the salt is present in an aqueous solution in a concentration ranging from 0.01 to 0.2 mole/liter.

43. Process according to Claim 42, characterized by the fact that the salt is present in an aqueous solution in a concentration ranging from 0.01 to 0.05 mole/liter.

44. Process according to Claims 1 - 3, characterized by the fact that water is used as the elution agent.

45. Process according to Claims 1 - 44, characterized by the fact that the membrane has a pore diameter of 0.05 to 20μm, preferably 0.2 to [omission] μm, most preferably 0.45 to 5.0 μm.

46. Use of a surface or a membrane made of material of a hydrophilic material whose surface has been made hydrophobic to isolate the nucleic acids.

47. Use according to Claim 46, characterized by the fact that the surface or membrane consists of nylon, polysulfone, polyether sulfone, polycarbonate, polyacrylate and acrylic acid copolymers, polyurethanes, polyamids, polyvinyl chloride, fluorocarbonates, polytetrafluoroethyl, polyvinylene difluoride, ethylene co-tetrafluoroethylene, polyethylene chlorodifluoroethylene copolymerisates or polyphenylene sulfide.

48. Use according to Claim 46, characterized by the fact that the membrane is a hydrophobisized nylon membrane.

49. Use according to any of Claims 46 to 48, characterized by the fact that the surface or membrane

is a hydrophilic surface or membrane which has been covered with a hydrophobic coating agent from the group of paraffins, waxes, metallic soaps, if desired with the addition of aluminum or zirconium salts, quaternary organic compounds, urea derivatives, lipid-modified melamine resins, silicones or zinc-organic compounds or with glutaric dialdehyde.

50. Mechanism comprising a hydrophobic surface according to any of Claims 1 to 3, a solution to immobilize the nucleic acid, a washing solution and, if desired, an elution solution.

Summary

This invention concerns a new process for the isolation and purification of nucleic acids, using hydrophobic surfaces and, in particular, hydrophobic membranes.